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2-AMINO-dA FORMS A MORE STABLE BASE PAIR WITH T

2-Amino-dA

As shown in Figure 1, the simplest approach to improving primers is to substitute A sites with 2-amino-A which forms three hydrogen bonds with T on hybridization to be analogous to G-C base pairs. 2-Amino-A also destabilizes A-G wobble mismatches, thus increasing specificity. Although 2-amino-dA monomers have been commercially available, they have had two severe drawbacks: the protection scheme and the cost. Because 2-amino-dA is very susceptible to depurination during the acidic deblocking step of DNA synthesis, mild deprotecting groups like PAC to protect both amino groups should not be used. The combination of N2-isobutyryl and N6-formamidinium protecting groups in our earlier monomer stabilized the monomer to depurination but made it very slow to deprotect, requiring 7 days in ammonium hydroxide at 55° or 17 hours at 55° in AMA for complete removal. After a significant development effort, we are happy to announce a new 2-amino-dA monomer (1) which appears to solve most of the earlier problems: deprotection is quite fast and effective in ammonium hydroxide and AMA; it is stabilized to depurination during synthesis; and the cost is only about 20% of the earlier monomer.

Recommended Usage Conditions

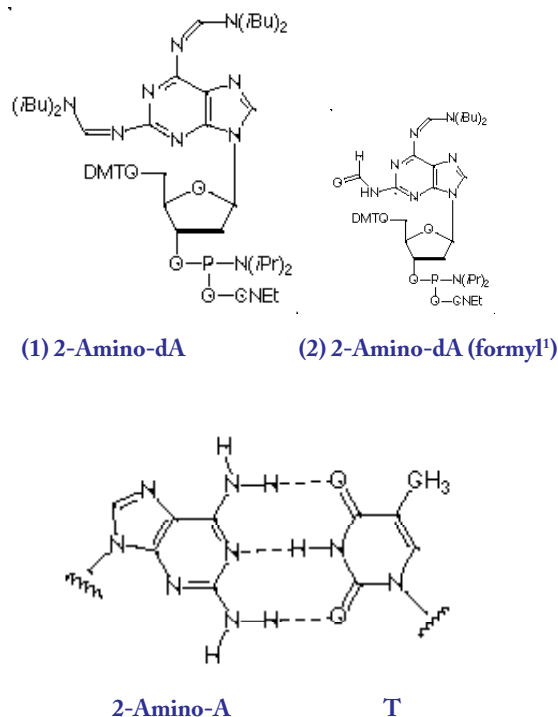
Regular conditions are used with the following exceptions.

	<i>Acceptable Conditions</i>	<i>Best Conditions</i>
<i>Coupling</i>	0.45M tetrazole in acetonitrile 15 minutes	0.25M DCI in acetonitrile 15 minutes
<i>Deblocking</i>	3% TCA in dichloromethane	2.5% DCA in dichloromethane (for a minimum of 60 seconds per cycle)
<i>Deprotection</i>	ammonium hydroxide 24h/55°	AMA 1.5h/65° 17h/RT

Note:

This phosphoramidite may contain varying amounts of the formyl analogue (2)¹, which is observed as a faster eluting peak in the RP HPLC of 2-amino-dA CE Phosphoramidite. Oligonucleotides synthesized with either form of 2-amino-dA CE Phosphoramidite yield identical products that co-elute on RP HPLC, have identical UV/Vis spectra, and give identical retention times when digested and analyzed for base composition.

FIGURE 1: STRUCTURES



(1) S. Vincent, C. Mioskowski, and L. Lebeau, *J Org Chem*, 1999, **64**, 991-997.

FIGURE 1: RP HPLC OF A CRUDE DMT-ON OLIGO CONTAINING THREE 2-AMINO-dA RESIDUES

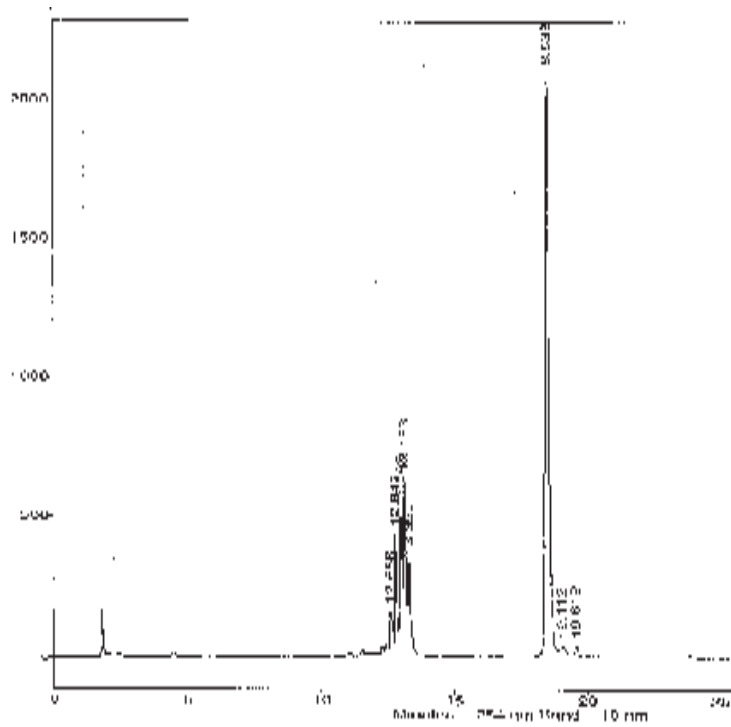


Figure 1

Synthesis was carried out using the standard reagents on a 1 micromole synthesis scale. DMT-on oligo was cleaved and deprotected with AMA overnight at 55°. Analysis is by RP HPLC on a Spherisorb ODS2 column.

Figure 2

Synthesis was carried out using the standard reagents with the exceptions that the activator was 0.25M DCI, deblocking with 2.5% DCA, on an LV200 synthesis cycle. DMT-off oligo was cleaved and deprotected with ammonium hydroxide 24h at 55°.

Figure 3

UV spectrum and RP HPLC of HO-(2-amino-dA)₂-Spacer C3.

FIGURE 2: RP HPLC OF A CRUDE OLIGO CONTAINING THREE 2-AMINO-dA RESIDUES USING BEST CONDITIONS

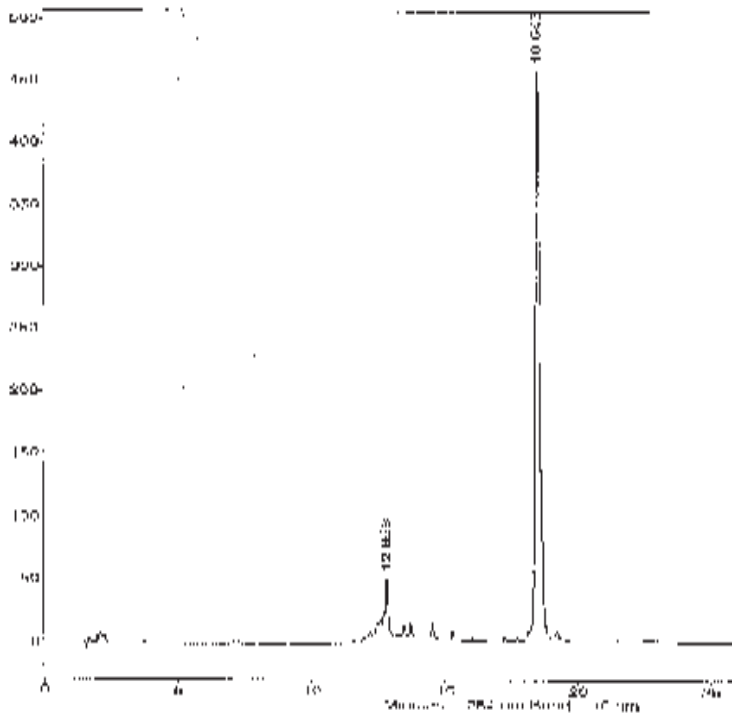


FIGURE 3: UV SPECTRUM OF 2-AMINO-dA

